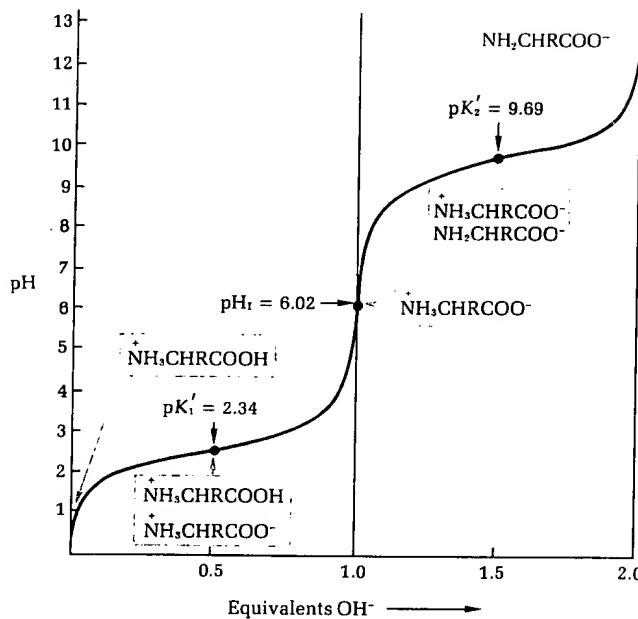


EXHIBIT B

Figure 4-6

Titration curve of alanine. The predominant ionic species at each cardinal point in the titration is given in boxes.



The acid-base behavior of the common amino acids is most simply formalized in terms of the Bronsted-Lowry theory of acids and bases (Chapter 2). A simple mono-amino monocarboxylic α -amino acid such as alanine is a dibasic acid in its fully protonated form; it can donate two protons during its complete titration with a base. The course of such a two-stage titration with NaOH can be represented in the following equations, which indicate the nature of each ionic species involved:

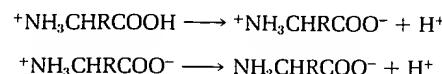


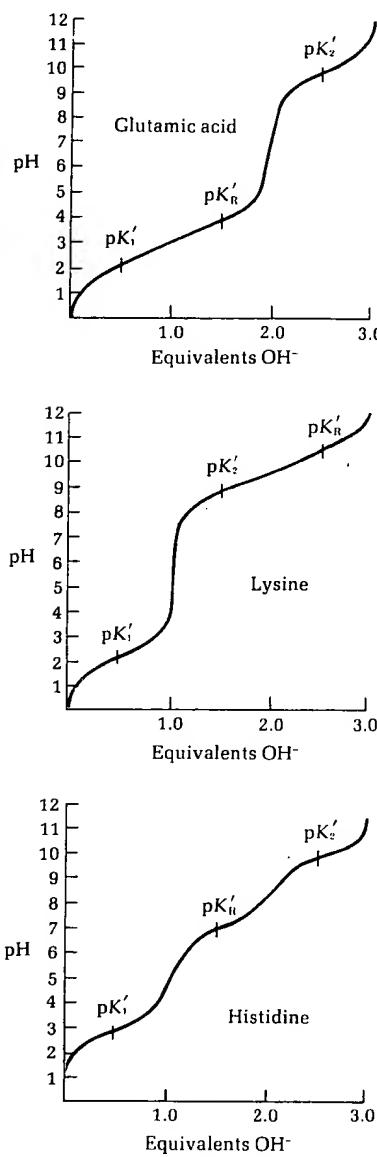
Figure 4-6 shows the biphasic titration curve of alanine. The pK' values of the two stages of dissociation are sufficiently wide apart to yield two clearly separate legs. Each leg has a midpoint where there is minimal change in pH as increments of OH^- are added. The apparent pK' values for the two dissociation steps may be extrapolated from the midpoints of each step; they are $\text{pK}'_1 = 2.34$ and $\text{pK}'_2 = 9.69$. At pH 2.34, the midpoint of the first step, equimolar concentrations of proton donor (${}^+\text{NH}_3\text{CHRCOOH}$) and proton acceptor (${}^+\text{NH}_3\text{CHRCOO}^-$) species are present. At pH 9.69, equimolar concentrations of ${}^+\text{NH}_3\text{CHRCOO}^-$ and $\text{NH}_2\text{CHRCOO}^-$ are present. Each of the two legs of the biphasic curve can be expressed mathematically to a very close approximation by the Henderson-Hasselbalch equation (Chapter 2); this means that we can calculate the ratios of ionic species at any pH, given the values for pK'_1 and pK'_2 .

Table 4-2 The pK' values for the ionizing groups of some amino acids (25°C)

	$\text{pK}'_{\alpha\text{-COOH}}$	$\text{pK}'_{\alpha\text{-NH}_3^+}$	pK'_R
Glycine	2.34	9.6	
Alanine	2.34	9.69	
Leucine	2.36	9.60	
Serine	2.21	9.15	
Threonine	2.63	10.43	
Glutamine	2.17	9.13	
Aspartic acid	2.09	9.82	3.86
Glutamic acid	2.19	9.67	4.25
Histidine	1.82	9.17	6.0
Cysteine	1.71	10.78	8.33
Tyrosine	2.20	9.11	10.07
Lysine	2.18	8.95	10.53
Arginine	2.17	9.04	12.48

Figure 4-7

Titration curves of glutamic acid, lysine, and histidine. In each case, the pK' of the R group is designated pK'_R .



for the ionizing
5°C)

pK'_1	pK'_2	pK'_3	R group
1.6			
1.69			
1.60			
1.15			
1.43			
1.13			
1.82	3.86		
.67	4.25		
.17	6.0		
.78	8.33		
.11	10.07		
.95	10.53		
.04	12.48		

At pH 6.02, there is a point of inflection between the two separate legs of the titration curve of alanine. There is no net electrical charge on the molecule at this pH, and the molecule will not move in an electrical field. This is the **isoelectric pH** (symbolized pH_I), which is the arithmetic mean of pK'_1 and pK'_2 , that is, $pH_I = 1/2(pK'_1 + pK'_2)$. These relationships are sufficiently exact for most purposes. However, a small but finite amount of the uncharged form ($\text{NH}_2\text{CHRCOOH}$) is also present in equilibrium with the charged forms. All monoamino monocarboxylic acids show essentially the same behavior.

Table 4-2 gives the pK' values for the ionizing groups of some amino acids. A number of important generalizations follow from these data:

- 1 The carboxyl group of monoamino monocarboxylic acids is a stronger acid than the carboxyl group of comparable aliphatic acids such as acetic acid ($pK' = 4.76$) and lactic acid ($pK' = 3.86$). Presumably, the nearby positively charged amino group tends to repel the positively charged H^+ of the carboxyl group and thus increase its tendency to dissociate.
- 2 The α -amino group of monoamino monocarboxylic acids is a stronger acid (or weaker base) than the amino group of comparable aliphatic amines.
- 3 All the monoamino monocarboxylic amino acids having uncharged R groups have nearly identical pK'_1 values and nearly identical pK'_2 values.
- 4 None of the monoamino monocarboxylic amino acids has significant buffering capacity at the physiological pH zone, that is, pH 6.0 to 8.0. They do show buffering capacity in the zones near their pK' values, i.e., the zones pH 1.3 to 3.3 and pH 8.6 to 10.6. There is only one amino acid with significant buffering capacity at pH 6 to 8, namely, histidine.
- 5 The β -carboxyl group of aspartic acid and the γ -carboxyl group of glutamic acid, although fully ionized at pH 7.0, have pK' values that are considerably higher than the pK' values of α -carboxyl groups and more nearly equal to that of simple carboxylic acids such as acetic acid.
- 6 The thiol or sulphhydryl group ($-\text{SH}$) of cysteine and the β -hydroxy group of tyrosine are only very weakly acidic. At pH 7.0, the former is about 8 percent ionized and the latter about 0.01 percent ionized.
- 7 The ϵ -amino group of lysine and the guanidine group of arginine are strongly basic; they lose their protons only at a very high pH. At pH 7.0, these amino acids have a net positive charge.

The titration curves of amino acids with R groups that ionize (such as histidine, lysine, and glutamic acid) are complex since the curve corresponding to the R group dissociation is superimposed on the curves for the α -amino and α -carboxyl groups (Figure 4-7).

Formol Titration

Formaldehyde in excess readily combines with the free (i.e., unprotonated) amino groups of amino acids to give methylol derivatives. This reaction causes an isoelectric